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PHYSICO CHEMICAL ANALYSIS OF PRACHANDABHAIRAVA RASA

[1]Dr. Arati Ludabude, [2]Dr. Prakash R. Deshpande.
[1]PG Scholar, [2]Professor & HOD, Department of Rasashastra and Bhaishajya Kalpana,
BVVS Ayurved Medical College and Hospital Bagalkot, Karnataka, India.

ABSTRACT

Physico-chemical analysis of any drug should be carried out before experimental and clinical trials. Chemical study ensures not only chemical constituents but also will tell us standard of any preparation. It not only gives standards of the product but also indirectly gives suggestions for further advancement if required. Various physico chemical tests were carried out to know moisture content, Ash value, Alcohol Soluble Extractive value, Water insoluble Ash and their respective values. X-ray diffraction (XRD) ofPrachandabhairava Rasa[1] enables us to know the finger print characterization of crystalline materials and the determination of their structure. Size and shape of unit cell for any compound can be detected most easily using x-raydiffraction. Scanning Electron Microscopy (SEM) was carried out to obtain micro-structural films at two different magnifications.High Performance Thin Layer Chromatography (HPTLC)analysis is to confirm the identity, quality and purity of a drug.

KEYWORDS: Prachandabhairava Rasa, Physico-chemical study, Instrumental Analysis.

INTRODUCTION

Ayurvedic drugs are time tested for their efficacy and need no validation for administration to patients. But in present scientific era, there is change in the mindset of patients. The word 'analyze' means to examine methodically and in detail for the purposes of explaination and interpretation. The Rasoushadhi's mentioned in Ayurvedic Pharmacopoeia need to be analyzed for physico-phyto chemically and instrumentally to confirm genuinity and safety before administration in human beings. Hence it is compulsory to describe a product by analytical standards that reveal quality, authenticity and purity of the product.

Safety of the drug to be administered is as par with its efficacy. Analytical study is mandatory to check the raw samples, intermediary products and final product. The presence of free metal or particles of large size in any formulation can lead to damage of vital organs of body. Hence highly sensitive modern parameters are employed for gaining information about identity, form, particle size and structure of contents of the formulation. The analytical studyPrachandabhairava Rasa was carried out under six different headings Organoleptic study, Test for Perfectness of *Kajjali***,** Physico-chemical Analysis, Preliminary Phytochemical Analysis, Qualitative analysis

by instrumental techniques like High Performance Thin Layer Chromatography (HPTLC), X-RAY Diffraction Studies (XRD), Scanning Electron Microscope and Energy Dispersive X-Ray Spectroscopy (SEM-EDX).

Preparation of Prachandabhairava Rasa[1]:

S. N	Sanskrit Name	English Name/ Latin Name	Quantity	Parts used
1	*Shuddha Kasisa*	*Ferrous sulphate* ($FeSO_4,7H_2O$)	60gms	-
2	*Shuddha Gandhaka*	*Sulphur*(S)	60gms	-
3	*Shuddha Parada*	*Hydrargyrum* (Hg)	60gms	-
4	*Shuddha Hingula*	*Mercuric sulphide* (HgS)	60gms	-
5	*Madhukapushpa*	*Madhuca longifolia* (Koen)	60gms	*Puspha*
6	*Guduchi*	*Tinospora cardifolia* (Willd)	60gms	*Kandha*
7	*Shalmali*	*Salmalia malabarica* Schott	60gms	*Moola*
8	*Dhanyaka*	*Coriandum sativum* Linn.	60gms	*Beeja*
9	*Bhunimba*	*Swertia chirata Buch Ham.*	60gms	*Panchanga*
10	*Devadaru*	*Cedrus deodara* (Roxb.) Loud.	60gms	*Kaashta*
11	*Tumburu*	*Zanthoxylum alatum* Roxb	60gms	*Phala*
12	*Tila*	*Sessamum indicum* D.C	60gms	*Beeja*
13	*Mudga*	*Phaseolus aureus* Roxb	60gms	*Beeja*
14	*Patola*	*Trichosanthes cucumerina* Linn.	60gms	*Moola*
15	*Draksha*	*Vitis vinifera*(Linn)	60gms	*Phala*
16	*Kushmanda bhasma*	*Benincasa hispida* (Thunb.)	60gms	*Beeja*
17	*Saireyaka*	*Barleria prionitis* Linn	60gms	*Panchanga*
18	*Kumari*	*Aloe vera*	60gms	*Patra*
19	*Bharangi*	*Clerodendrum serratum* (Linn.)	60gms	*Moola*
20	*Bala*	*Sida cardifolia* Linn	60gms	*Moola*
21	*Atibala*	*Abution indicum*(Linn)	60gms	*Moola*
	Prachandabhairava Rasa		1270gms	

Kajjali is prepared out of mixing Shuddha Parada, Shuddha Gandhaka and to this kajjali added Shuddha Hingula, Shuddha Kasisa. Then to PBR Kajjali added one after the other above mentioned churna's. Trituration was done till the homogeneous mixture. Then, with the help of ghee and Madhu prepared 1 masha size vati's. Then dried under the shade and stored in airtight container.

Table No 1: Organoleptic test results of Prachandabhairava Rasa.

Sample	Colour	Odour	Form	Taste
Prachandabhairava Rasa	Blackish brown	Disagreeable	Powder	Not appreciated

Table No 2: Test of Perfectness of Kajjali[2]

Test	Observation and Result
Nischandratva	The Kajjali was observed in bright sunlight. It was not having any luster – Positive
Rekhapurnatva	The Kajjali was rubbed in between index finger and thumb. It penetrates the

	furrows of the fingers – Positive
Varitaratva	A small amount of Kajjali was carefully sprinkled over the surface of a beaker contained a stagnant water. It was found that total portion of kajjali was floating on the water surface – Positive
Unam	A small amount of Kajjali was carefully sprinkled in beaker full of water and a grain is placed on the floating matter It was found that the grain was floating on the water surface – Positive

Table No 3: Physico Chemical analysis of Prachandabhairava rasa[3,4,5,6,7,8]:

Analytical Test	Result
Moisture content	1.2%
Total ash	17%
Acid insoluble ash	5%
Aqueous extract	21.6%
Alcoholic extract	12%
pH	4.29

TableNo4: Preliminary Phytochemical analysis of Prachandabhairava Rasa[9].

S.N	Test	Water	Alcohol
1	Test for Carbohydrates	Positive	Positive
2	Test for Reducing sugar	Positive	Positive
3	Test for Proteins	Negative	Negative
4	Test for Amino Acids	Negative	Negative
5	Test for Steroids	Positive	Positive
6	Test for Saponins	Negative	Negative
7	Test for Alkaloids	Positive	Positive
8	Test for Tannins and Phenolic compounds	Positive	Positive
Test for Glycosides			
9	Test for Cardiac Glycosides	Negative	Positive
10	Test for Anthraquinone Glycosides	Negative	Positive
11	Test for Saponin Glycosides	Negative	Negative

Table No: 5- Inorganic element analysis of Prachandabhairava Rasa[10]:

S. N	Test	Water	Alcohol
1	Calcium	Positive	Positive
2	Zinc	Negative	Negative
3	Sodium	Positive	Positive
4	Potassium	Positive	Positive
5	Iron	Negative	Negative
6	Sulphate	Positive	Positive
7	Aluminium	Negative	Negative
8	Chloride	Negative	Negative
9	Carbonate	Negative	Negative
10	Ammonium salts	Negative	Negative
11	Barium salts	Negative	Negative

Table No6: Showing the result of analysis of vati[11]

S.N	Parameters	Results

1	Disintegration	35min.
2	Hardness	5.75kg/cm²
3	Friability	0.41%

INSTRUMENTAL ANALYSIS:

1. X- Ray Diffraction Studies[12]:

The discovery of X-rays in 1895AD created a milestone in medical history. X-ray diffraction has been in use in two main areas, for the finger print characterization of crystalline materials and thedetermination of their structure. Each crystalline solid has its unique characteristic X-ray powder pattern which may be used as a "finger print" for its identification.

Present Study:

Present crystallographic study was carried out on Prachandabhairava Rasa.

Materials used

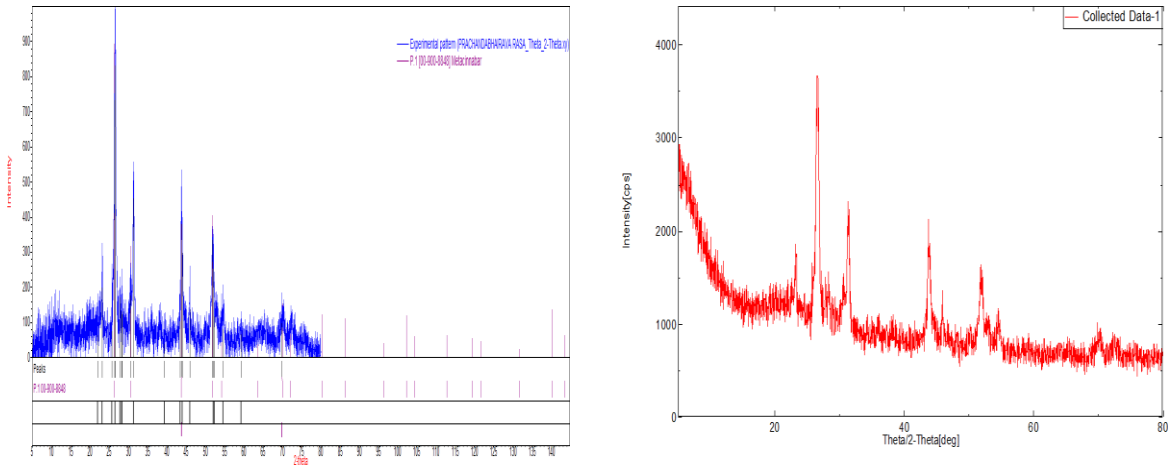
1. XRDPallet Maker
2. Glass slides
3. Glass XRD holders
4. 1 gm of sample (Prachandabhairava rasa)

Instrument make & model: Rigaku smart lab s200

Table No-7: Steps -procedure

Steps	Procedure
1	1 gm of PBRasa air-dried samples was accurately weighed and loaded on glass slide
2	XRAY_CHAR = K-ALPHA *WAVE_LENGTH1= 1.54059 *WAVE_LENGTH2= 1.54441
3	2theta/theta = 5 to 80 Degree
4	XRD analysis is performed using Rigaku Instrument with 2Avg measurement points to get least standard deviation.

Fig.1: XRD Analysis of Prachandabhairava Rasa



00-900-8848

COD CIF File	http://www.crystallography.net/cod/9008848.html
Mineral Name	Metacinnabar
Formula	Hg S
Quality	C (calculated pattern)
I/Ic	26.03
Reference	Wyckoff, R. W. G., Crystal Structures, **1** (1963)
Space Group	F -4 3 m (216)
Crystal system	Cubic
Cell parameters	a=5.8517 Å
Cell volume	200.38 Å3
Wavelength	1.54056 Å
μ(Cu Kα) 1345.964	cm-1

Diffraction data

2theta	d[A]	Int.	hkl	mult
26.3581	3.3785	1000.00	1 1 1	8
30.5285	2.9258	355.40	2 0 0	6
43.7168	2.0689	483.54	2 2 0	12
51.7702	1.7644	452.39	3 1 1	24
54.2590	1.6892	97.70	2 2 2	8
63.5444	1.4629	72.04	4 0 0	6
70.0264	1.3425	171.21	3 3 1	24
72.1259	1.3085	122.07	4 2 0	24
80.3097	1.1945	136.11	4 2 2	24
86.3083	1.1262	124.00	5 1 1	24
96.2604	1.0344	43.35	4 4 0	12
102.2959	0.9891	133.23	5 3 1	48
104.3314	0.9753	67.22	6 0 0	6
112.7242	0.9252	71.40	6 2 0	24
119.3455	0.8924	61.19	5 3 3	24
121.6501	0.8822	52.15	6 2 2	24
131.5680	0.8446	25.38	4 4 4	8
140.1211	0.8194	151.01	7 1 1	24
143.3197	0.8115	69.53	6 4 0	24

Physical Properties

Calc. density	7.712 g cm-3

Remarks

Diffraction pattern calculated by EXPO from COD database CIF file
I/Ic calcuted by EXPO

2. Scanning Electron Microscopy (SEM) & Energy Dispersive X-Ray Spectroscopy (EDS)[13]:

Working Principle:

Electron microscopes have a greater resolving power than a light-powered optical microscope, as electrons have wavelengths about 100,000 times shorter than visible light (photons), and can achieve better than 50m resolution and magnifications of about 10,000,000X, whereas ordinary, non-cofocal light microscopes are limited by diffraction to about 200 nm resolution and magnifications below 2000x.4

Present study:

The present study was carried out on Prachandabhairava Rasa

Materials used

1. SEM samples holder cu studs
2. Ultra Sonicator
3. Dryer
4. 0.5 gm of sample

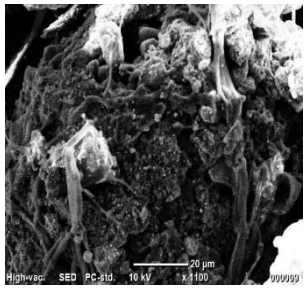
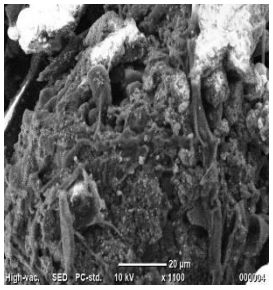
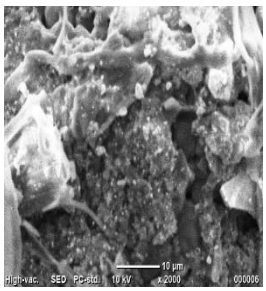
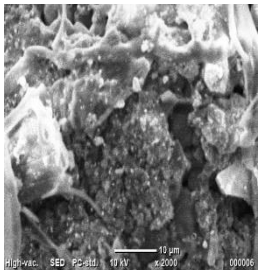
Instrument make & model: jeol - eds s2000

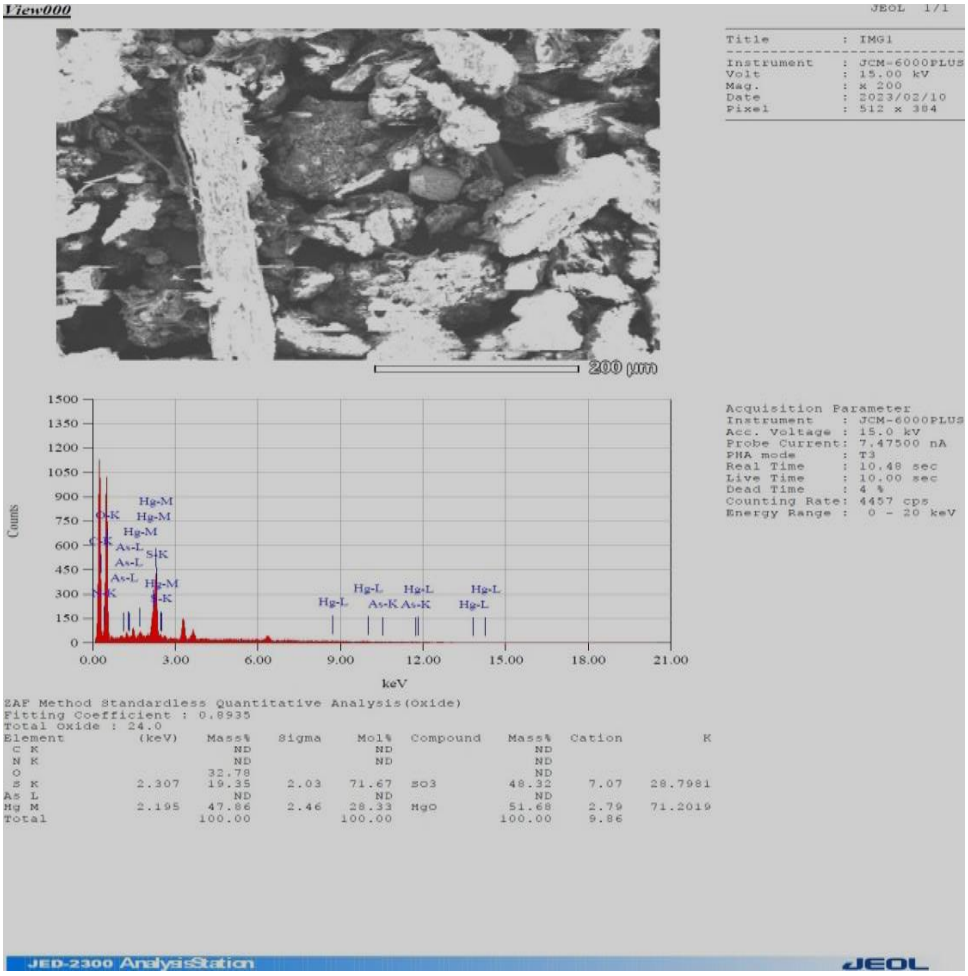
Table No-7: Steps and procedure:

Steps	Procedure
1	0.5 gm of air-dried samples was accurately weighed.
2	Samples were diluted with 2 ml Ethanol in a clean glass beaker.
3	Samples were ultra sonicated for 30 min to obtain proper particle dispersion and transferred to copper studs and air dried to remove moisture.
4	SEM-EDAX analysis is performed using JEOL_SEM with different magnification and EDS Elemental spectra as requested.

Fig.2: SEM-EDS Analysis of Prachandabhairava Rasa

SEM MORPHOLOGY



HPTLC (High Performance Thin Layer Chromatography)[14]:-

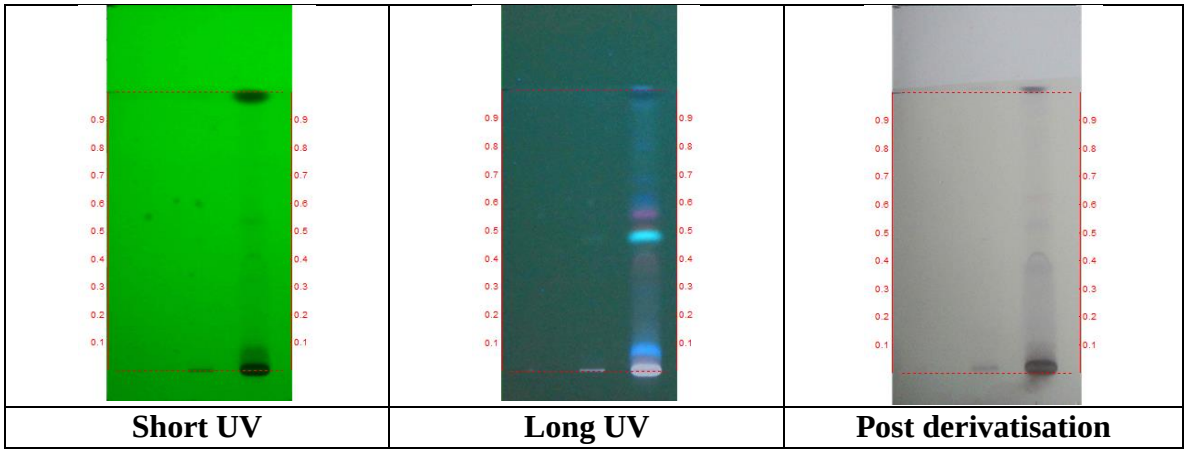
The technique through which the chemical components present in complex mixtures are separated, identified, and determined is termed as chromatography. It is the technique in which the components of a mixture are separated based upon the rates at which they are carried or moved through a stationary phase (column) by a gaseous or liquid mobile phase. HPTLC is sophisticated/enhanced form of thin layer chromatography (TLC). It has same principle as that of TLC i.e., adsorption and partition methods. This technique is widely used in many fields both for qualitative and quantitative estimation of a mixture. Russian Botanist *Mikhail Tswett* used the technique to separate various plant pigments.

Procedure:

1g of Prachandabhairava rasa sample was kept in 20.ml of ethanol macerated at room temperature with intermittent soaking, filtered. 3, 6, 9µl of each of the above extract was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl Acetate (9.0: 1.0). The developed plates were visualized in short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and

620nm (Post derivatisation). R_f, colour of the spots and Densitometric scan were recorded.

Fig.3:HPTLC Analysis of Prachandabhairava Rasa



Short UV	Long UV	Post derivatisation

Track 1 - **Prachandabhairava rasa**– 3µl

Track 2 - **Prachandabhairava rasa**– 6µl

Track 3 - **Prachandabhairava rasa**– 9µl

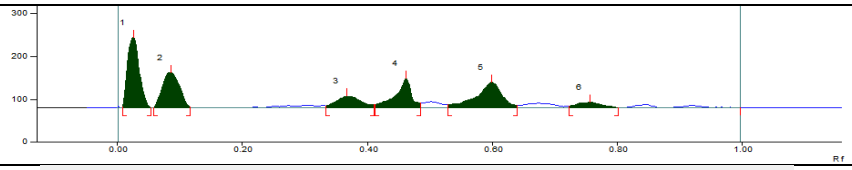
Solvent system – **Toluene: Ethyl Acetate (9.0: 1.0)**

Table No-8:R_f values of sample of Prachandabhairava rasa

Short UV	Long UV	Post derivatisation
-	0.07 (F. blue)	-
0.40 (Green)	0.40 (F. blue)	-
-	0.49 (F. blue)	-
0.54 (Green)	0.54 (F. blue)	0.53 (Purple)
-	0.56 (F. blue)	-
-	0.62 (F. blue)	0.62 (Purple)
-	0.68 (F. blue)	-
-	0.81 (F. blue)	-
-	-	0.87 (Purple)

***F – Fluorescent; L –Light; D – Dark**

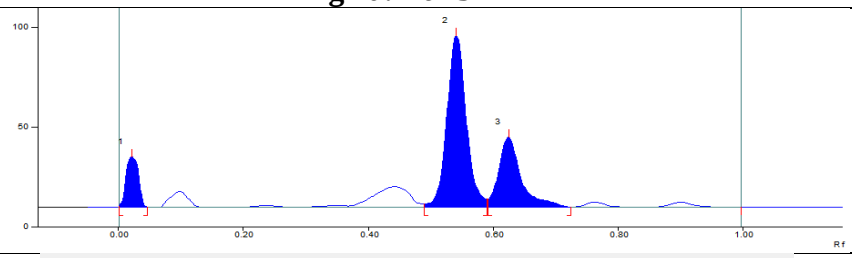
Figure 4. Densitometric scan of Prachandabhairava rasa



Track 3, ID: Prachandabhairva rasa

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	2.4 AU	0.03 Rf	163.8 AU	39.58 %	0.05 Rf	0.8 AU	2221.5 AU	27.86 %
2	0.06 Rf	0.8 AU	0.09 Rf	82.1 AU	19.83 %	0.12 Rf	1.2 AU	1622.9 AU	20.35 %
3	0.33 Rf	4.6 AU	0.37 Rf	27.1 AU	6.56 %	0.41 Rf	6.9 AU	782.2 AU	9.81 %
4	0.41 Rf	7.3 AU	0.46 Rf	68.7 AU	16.60 %	0.49 Rf	11.1 AU	1233.6 AU	15.47 %
5	0.53 Rf	7.5 AU	0.60 Rf	59.3 AU	14.32 %	0.64 Rf	5.6 AU	1745.6 AU	21.89 %
6	0.72 Rf	4.5 AU	0.76 Rf	12.9 AU	3.12 %	0.80 Rf	0.1 AU	367.5 AU	4.61 %

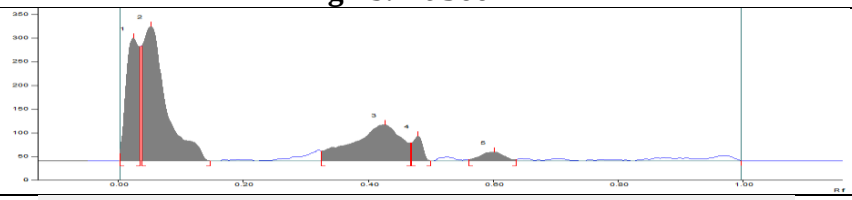
Fig 4a. At 254nm



Track 3, ID: Prachandabhairva rasa

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.6 AU	0.02 Rf	25.3 AU	17.26 %	0.05 Rf	0.1 AU	365.4 AU	11.50 %
2	0.49 Rf	1.6 AU	0.54 Rf	86.1 AU	58.73 %	0.59 Rf	4.1 AU	1908.6 AU	60.05 %
3	0.59 Rf	4.1 AU	0.63 Rf	35.2 AU	24.01 %	0.73 Rf	0.1 AU	904.2 AU	28.45 %

Fig 4b. At 366nm



Track 3, ID: Prachandabhairva rasa

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	15.4 AU	0.02 Rf	259.7 AU	37.61 %	0.04 Rf	41.5 AU	4024.6 AU	23.62 %
2	0.04 Rf	242.8 AU	0.05 Rf	283.7 AU	41.09 %	0.15 Rf	0.1 AU	7678.5 AU	45.07 %
3	0.33 Rf	21.1 AU	0.43 Rf	76.2 AU	11.03 %	0.47 Rf	37.9 AU	4213.7 AU	24.73 %
4	0.47 Rf	38.2 AU	0.48 Rf	52.2 AU	7.57 %	0.50 Rf	0.3 AU	600.0 AU	3.52 %
5	0.56 Rf	2.9 AU	0.60 Rf	18.6 AU	2.70 %	0.64 Rf	3.0 AU	521.6 AU	3.06 %

Fig 4c. At 620nm (Post derivatisation)

DISCUSSION:

The prepared Prachandabhairava Rasa was blackish brown powder with disagreeable smell. Prachandabhairava Rasa showed 1.2%, loss on drying with 12%. total ash value.Ashwas higher side since more of herbal drugs are constituents. Acid insoluble ash value was 2.5%. Extractive value of an aqueous extract was 21.6% and alcoholic was 12%. Here an aqueous extractive value is more when compared to alcoholic extract as this formulation is having more herbal drugs. Herbal drugs are more soluble in water rather than alcoholic. PBR has 4.29 pH which indicates compound is acidic in nature.

Organic compounds like Alkaloids, Tannins, steroids, carbohydrates were present in both of aqueous and alcoholic extract of PBR. Inorganic compounds like Ca, Na, K, SO_4 were present in both of aqueous and alcoholic extract of PBR.

X-RAY Diffraction Study of PBR sample revealed crystal structures as The diffraction showed the peak of mercury which was in HgS form. The given sample was cubic in nature. The humps in the diffractogram and absence of sharp peaks suggest that the original compound has been transformed into some low crystalline or amorphous like substance probably due to heat treatment.

Peaks at intervals showed the traces of Hg (Mercury), S (Sulphur). Here Hg and S are from kajjali which was used as base for preparing of Prachandabhairava Rasa even hingula (HgS) is also used. The analysis showed the traces of all the elements which are the components of Prachandabhairava rasa. Elements mass% present in Prachandabhairava rasaO (32.78%), S (19.35%) and Hg (47.86%).

In HPTLC study,3 tracks have been used with PBR sample 3µl, 6µl and 9µl in respective 3 tracks. In track I (SHORT UV), 2 Rf value, track II (LONG UV), 8 Rf values and in track III (Postderivatisation) 3 Rf values were detected. Detected same Rf values like 0.40, 0.54 in two tracks in SHORT UV and LONG UV. Then same Rf value was detected 0.62 in Post derivatisation. In Densitometric scan at wavelength of 254nm,366nm and 620nm got the peaks 6,3,5 respectively.

CONCLUSION:

Prachandabhairava Rasa is a Kharaleeya rasayana which is mentioned in Rasa Ratnakara indicated in Apasmara. Ingredients of Prachandabhairava Rasa are readily available, easy to prepare and cost effective. The standard analytical parameters of Prachandabhairava Rasa are not available in API and AFI. Hence this analytical study of Prachandabhairava Rasa is an attempt towards monograph of the same. Analytical findings of Prachandabhairava Rasa are consistent with ingredients.

REFERENCES:

1. Swaminath Mishra; Rasa Ratnakara, Hindi vyakhya, Choukambha publishers, Varanasi, Edition 2003, Chikitsa khanda, Shloka number 28-32. Page Number 348.

2. Acharya Vagbhata; Rasa Ratna Samucchaya with Rasa prabha Hindi vyakhya by Dr. Indradev Tripathi. Chaukambha Sanskrit Samsthana, Varanasi, Edition 1998.8th chapter. Shloka number:25-27 Page Number: 90-91.

3. Ayurvedic Pharmacopoeia of India; Part 1, Volume IX, e-book, Appendix volume(s)-1,2,3,4, Page 113, 114, 130 -132 Government of India, Ministry of Health and Family welfare, Department of AYUSH 2007.
4. Ayurvedic Pharmacopoeia of India; Part 1, Volume IX, e-book, Appendix volume(s)-1,2,3,4, Page 113, 114, 130 -132 Government of India, Ministry of Health and Family welfare, Department of AYUSH 2007.
5. Ayurvedic Pharmacopoeia of India; Part 1, Volume IX, e-book, Appendix volume(s)-1,2,3,4, Page 113, 114, 130 -132 Government of India, Ministry of Health and Family welfare, Department of AYUSH 2007.
6. Ayurvedic Pharmacopoeia of India; Part 1, Volume IX, e-book, Appendix volume(s)-1,2,3,4, Page 113, 114, 130 -132 Government of India, Ministry of Health and Family welfare, Department of AYUSH 2007.
7. Ayurvedic Pharmacopoeia of India; Part 1, Volume IX, e-book, Appendix volume(s)-1,2,3,4, Page 113, 114, 130 -132 Government of India, Ministry of Health and Family welfare, Department of AYUSH 2007.
8. Ayurvedic Pharmacopoeia of India; Part 1, Volume IX, e-book, Appendix volume(s)-1,2,3,4, Page 113, 114, 130 -132 Government of India, Ministry of Health and Family welfare, Department of AYUSH 2007.
9. Ayurvedic Pharmacopoeia of India; Part 1, Volume IX, e-book, Appendix volume(s)-1,2,3,4, Page 113, 114, 130 -132 Government of India, Ministry of Health and Family welfare, Department of AYUSH 2007
10. Ayurvedic Pharmacopoeia of India; Part 1, Volume IX, e-book, Appendix volume(s)-1,2,3,4, Page 113, 114, 130 -132 Government of India, Ministry of Health and Family welfare, Department of AYUSH 2007
11. Ayurvedic Pharmacopoeia of India; Part 1, Volume IX, e-book, Appendix volume(s)-1,2,3,4, Page 113, 114, 130 -132. Government of India, Ministry of Health and Family welfare, Department of AYUSH 2007.
12. https://en.m.wikipedia.org/wiki/X-ray_scattering_techniques as on 10th May 2023,09Am
13. https://en.m.wikipedia.org/wiki/Scanning_electron_microscopy on 20th May 2023, 11am.
14. Dr Sethi P.D, HPTLC Quantitative Analysis of Pharmaceutical Formulations, 1st Edition 1996.

CORRESPONDING AUTHOR
Dr Arati Ludabude
Department of Rasashastra and Bhaishajya Kalpana, BVVS Ayurved Medical College and Hospital Bagalkot, Karnataka, India
Email: aratirl4584@gmail.com

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